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Dyskeratosis congenita: three additional families show linkage to a locus in Xq28

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Abstract

Dyskeratosis congenita (DC) is a rare inherited disorder with most families being of the X linked recessive type. We describe three families which show linkage to the marker DXS52 on Xq28. The combined maximum lod score was 2.00 at zero recombination. This is further evidence that the X linked DC gene is located at Xq28 and brings the reported maximum lod score for DC and DXS52 to 5.33 at zero recombination fraction, with a supporting recombination fraction interval of 0·00-0·10.

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Dyskeratosis congenita is a rare inherited disorder, characterised by reticulate skin hyperpigmentation, nail dystrophy, lacrimal duct obstruction, and leucoplakia of the mucous membranes. More serious features are bone marrow hypofunction, pancytopenia, and a predisposition to malignancy.

Manifestation is usually in childhood with serious complications starting in mid teens with a mean age of death at 23.6 years (range 8 to 50 years). Over 100 cases, mostly males (M/F ratio 8:1), have now been reported. About half of these are sporadic and half familial.[1]

X linked recessive inheritance is supported by pedigree pattern in several large families[2,3] but reports of five affected females in one family with a milder and less typical phenotype[4] and male to male transmission[5] suggest genetic heterogeneity.

Linkage analysis in one large pedigree using multiple X chromosomal DNA polymorphisms assigned the locus to Xq28.[6] This paper reports three further families which show linkage to this same region of the X chromosome.

Subjects and methods

PATIENTS (FIG 1)

Family 1 has been described in detail by Dokal *et al.*[7] The proband first presented with symptoms of DC at the age of 29 years. His younger brother was diagnosed when he was 26 years old, while being assessed as a potential bone marrow donor.

Patient 3 is a nephew of patients 1 and 2. He was investigated at the age of 12 years and the diagnosis of DC was made. An older half sister of the proband had two sons, who showed no signs of DC on clinical examination and laboratory investigation. Their phenotype was assigned as normal.

Family 2 is a South African family and has been described in detail by Jacobs *et al.*[8] Three boys and their maternal grandfather were diagnosed as having DC. The grandfather and two of the affected boys were dead, leaving one affected boy, his mother, and two normal brothers available for this linkage study.

In family 3 the proband, of British origin, was diagnosed as having DC. His uncle had died earlier of pneumonia and was noted to have nail dystrophy. The proband, his normal brother, and mother, who is an obligate carrier, were available for linkage analysis.

DNA STUDIES

DNA was extracted from leucocytes using standard methods and 5 to 10 μg of DNA were digested overnight using *Taq*I enzyme (NBL) under conditions defined by the manfacturer. Restriction fragments were separated in 0·8% agarose gels and transferred by Southern blotting[9] to Hybond–N (Amersham).

The probe used was DXS52 (St14.1),[10] which is multiallelic with a PIC value of 0·77, and is a reference marker for Xq28. The probe

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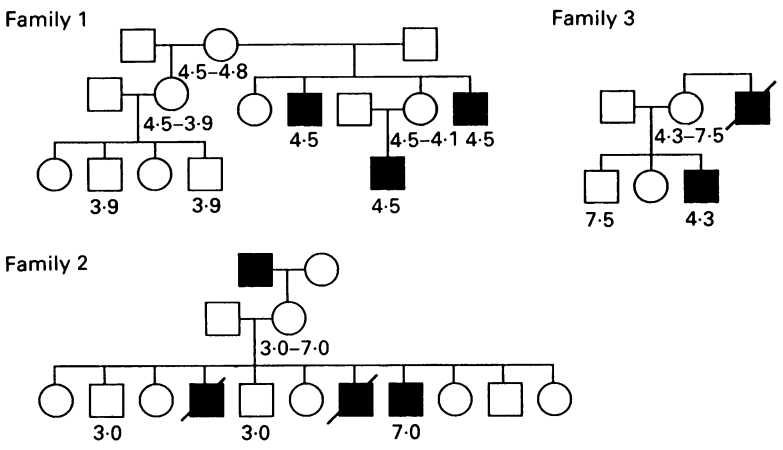

Pedigrees of the three dyskeratosis congenita families studied. DXS52 genotypes are indicated.

Table 1 Lod scores for dyskeratosis congenita and DXS52.

	Recombination fraction					
Family	0·00	0·05	0·10	0·20	0·30	0·40
1	1·10	0·98	0·84	0·54	0·34	0·14
2	0·60	0·55	0·46	0·32	0·17	0·05
3	0·30	0·26	0·22	0·13	0·06	0·02
Total	2·00	1·79	1·52	0·99	0·57	0·21

Table 2 Combined lod scores from this study and that of Connor et al[6] between dyskeratosis congenita and DXS52.

	Recombination fraction					
Study	0·00	0·05	0·10	0·20	0·30	0·40
Present	2·00	1·79	1·52	0·99	0·57	0·21
Connor *et al*[6]	3·33	3·06	2·77	2·14	1·43	0·70
Total	5·33	4·85	4·29	3·13	2·00	0·91

was labelled with ^{32}P either by nick translation[11] or by oligonucleotide labelling.[12] Hybridisation was performed overnight at 42°C in hybridisation buffer as follows: 50% formamide, 1 × Denhardt's, 5 × SSC, 20mmol/l NaH_2PO_4, pH 6·8, 0·1 mg/ml heat denatured salmon sperm DNA, 0·02 mg/ml poly(A), and 10% dextran sulphate. Non-specifically bound probe was removed by washing in 1 × SSC, 0·1% sodium dodecyl sulphate (SDS) followed by 0·5 × SSC, 0·1% SDS, both at room temperature, and finally one or two washes in 0·1 × SSC, 0·1% SDS at 65°C. Bands were visualised by autoradiography for one to seven days at −80°C using intensifying screens.

Lod scores were calculated using the computer program LIPED.[13] In these calculations a disease allele frequency of 0·01 was used, and complete penetrance was assumed for hemizygous males. The supporting interval was estimated by finding the recombination fraction at the lod score which was one unit lower than the maximum lod score.

Results

Pairwise linkage analysis showed no recombination between the locus for DC and RFLPs identified by DXS52 in all three families. A combined maximum lod score of 2·00 was observed at zero recombination fraction (table 1).

These results bring the total lod score for DC and DXS52 to 5·33 at zero recombination (table 2). The supporting recombination interval is 0·00–0·10.

Discussion

Linkage analysis in one large family using multiple X chromosomal DNA polymorphisms has previously assigned the gene for X linked recessive dyskeratosis congenita to Xq28.[6] Another family showed cosegregation of DC and G6PD deficiency[14] which also maps to Xq28. Only one recombination between DC and G6PD was observed among nine postpubertal males. This study was, however, limited as the assay only allowed the genotype of the females to be assigned on the basis of their offspring's phenotypes.

The present study supports tight linkage between DC and loci on Xq28 and raises the maximum lod score for DXS52 from 3·33 previously described[6] to 5·33 (table 2). These results will allow further testing of other kindreds to identify genetic heterogeneity which has been suggested on the grounds of clinical and pedigree data.

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